

Antagonist Test *Trichoderma* Sp. Tartar Isolate Against *Colletotrichum* Sp. Isolates Causes of Anthracnose In Chili Peppers (*Capsicum Frutescens* L.)

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Abstract

Anthracnose disease in chili peppers caused by *Colletotrichum* sp. is one of the diseases that can reduce the yield and quality of chili production. This study aims to test the ability of *Trichoderma* sp. Taratara isolate antagonists against *Colletotrichum* sp. in vitro, determine the viability of conidia, and observe the mechanism of antagonism that occurs. The research was conducted at the Advanced Biology Laboratory of FMIPA, Sam Ratulangi University using a laboratory experiment method with a Complete Random Design (RAL) consisting of dual culture control and treatment, five replicas each. *Trichoderma* sp. was explored from plantation soil in Taratara Village using the fishing method with coconuts, then purified and morphologically characterized. The viability test was carried out through observation of conidia germination after 24 hours of incubation, while the antagonist test was carried out by the dual culture method on PDA media for 7 days. The results showed that Taratara isolates had a conidia viability of $96.74 \pm 3.98\%$. The growth of *Trichoderma* sp. colonies took place faster than *Colletotrichum* sp., with the diameter of the colony on day 7 reaching 8.5 cm and 4.7 cm, respectively. The percentage of resistance of *Trichoderma* sp. against *Colletotrichum* sp. increased during the incubation period and reached 60.43% on day 7. Observations at the interaction zone showed that the mechanisms of antagonism that were actually observed were space and nutrient competition, characterized by faster and more media-dominated growth of *Trichoderma* sp. and an indication of mycoparasitism. These results show that *Trichoderma* sp. Taratara isolate has the potential to be a biological control agent against *Colletotrichum* sp. causing anthracnose in chili peppers.

Keywords: *Trichoderma* Sp., *Colletotrichum* Sp., Antagonism, Dual Culture, Anthracnose

Introduction

Chili pepper (*Capsicum frutescens* L.) is an economically important horticultural crop of the Solanaceae family, valued for its bioactive compounds such as capsaicin, capsaicinoids, and vitamin C (Polii et al., 2019). National chili production continues to rise in line with growing household and industrial demand, with major production centres in Java and outside Java together contributing 79.15% of national output

(Misqi *et al.*, 2020). One of the most damaging diseases is anthracnose, caused by the fungus *Colletotrichum sp.* The disease attacks leaves, stems, and fruits, producing dark-brown to blackish lesions that progress into soft or dry rot; in severe infections, fruits shrivel and rot entirely, sharply reducing yield and quality (Wang *et al.*, 2021).

Biological control agents offer a strategic solution in modern agriculture. Antagonistic fungi such as *Trichoderma sp.* act specifically, leave no harmful residues, and carry a lower risk of resistance development compared with chemical pesticides (Siahaan, 2020). *Trichoderma sp.* is well documented as an effective antagonist that suppresses pathogenic fungi through competition, antibiosis, mycoparasitism, and induction of plant resistance (Sundari *et al.*, 2014), and it also functions as a plant-growth enhancer that accelerates root and shoot development and increases yield (Setyadi *et al.*, 2017). Recent studies confirm the effectiveness of *Trichoderma sp.* against various plant pathogens, including on chili (Fardhani *et al.*, 2023) and shallot (Rahmadani *et al.*, 2021).

Local microbial isolates are of particular interest because they are naturally adapted to site-specific conditions of temperature, humidity, soil pH, and resident microbial communities (Amalia *et al.*, 2023), suggesting favourable survival and establishment in their environment of origin — although this adaptive advantage must still be verified experimentally. To date, no study has evaluated the antagonistic ability of a local *Trichoderma sp.* isolate from Taratara Village, North Sulawesi, against a *Colletotrichum sp.* isolate causing chili anthracnose. Although *Trichoderma sp.* has been extensively studied as a biological control agent, information on the *in vitro* antagonistic potential of this specific local isolate remains unavailable, which limits its use as a basis for developing a biocontrol agent suited to local agricultural conditions in North Sulawesi.

Method

This research was conducted from January to May 2026. Exploration of *Trichoderma sp.* was carried out in plantation soil in Taratara Village, West Tomohon District, Tomohon City, North Sulawesi. Inoculation, identification, and the antagonistic assay were carried out at the Advanced Biology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University.

The antagonism test was conducted using the dual-culture method on Potato Dextrose Agar (PDA) medium. The treatments consisted of P1 = *Trichoderma sp.* (Taratara isolate) paired with *Colletotrichum sp.* and P0 = control (*Colletotrichum sp.* without *Trichoderma sp.*). Petri dishes were incubated at room temperature ($27 \pm 2^\circ\text{C}$) for 7 days. Observations were made daily by measuring the diameter of *Colletotrichum sp.* colonies in centimeters (cm).

The percentage of *Trichoderma sp.*'s inhibitory effect on *Colletotrichum sp.* was calculated based on the diameter of the pathogen's colony growth using the formula according to Rahman *et al.* (2023):

$$\text{Inhibition} = (d1 - d2) / d1 \times 100\% \quad (1)$$

Description:

- d1 = Colony diameter of the pathogen in the control
- d2 = The diameter of the pathogen colony in the presence of *Trichoderma*.

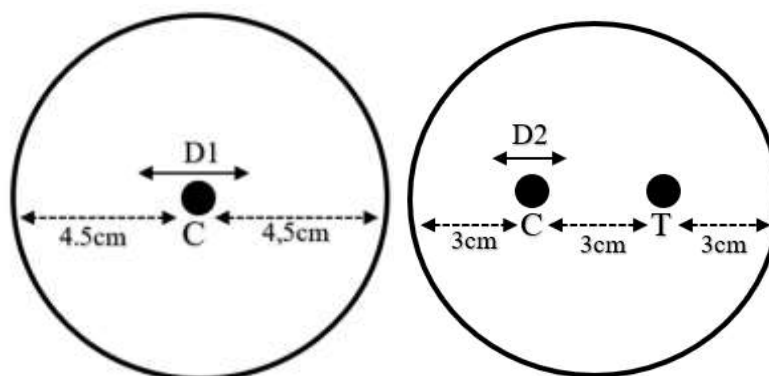


Figure 1. Antagonist test *Trichoderma* sp. tartar isolate against *Colletotrichum* sp. isolates causes of anthracnose in chili peppers (*Capsicum frutescens* L.)

Results and Discussions

Morphological characteristics of *Trichoderma* sp. Taratara and *Colletotrichum* sp.

Macroscopic observations of *Trichoderma* sp. Taratara isolates (Figure 2a) show a change in colony color from white on the first–second day, to greenish-yellow on the third to fifth day, then dark green on the sixth–seventh day. The colony is rounded in shape and develops until it meets the surface of the petri dish. Microscopically (Figure 2b), the isolates have hyaline, insulated, and branched hyphae, regularly branched conidiophores, and slightly tapered phylids at the tips of the branches. Conidia are rounded to almost spherical and oval, with a greenish color. Based on these macroscopic and microscopic characteristics, Taratara isolates are morphologically identified up to the genus level as *Trichoderma* sp.



Figure 2. Macroscopic (a) and microscopic (b) morphology of *Trichoderma* sp. Taratara isolation on PDA on day 7. Microscopic observations at 400× total magnification showed branched hyphae, conidiophores, phyalides, and conidae. (c) Microscopic morphology of *Trichoderma* sp. (Suanda, 2016) 1. conidiophore 2. conidiophore branch 3. phylidy 4. conidia/phialospore

The *Colletotrichum* sp. isolates used in this study were obtained from the North Sulawesi Food and Horticultural Crop Quality Protection and Testing Center (BPMPTH). *Colletotrichum* sp. colonies are circular in shape, with mycelium growth spreading radially from the inoculation point. The colony surface is dominated by cotton-colored white mycelium, with an orange to brownish-orange conidium mass in the center and a beige to light brown color at the edges; the colony edges are flat and the growth pattern is relatively symmetrical, reaching a diameter of about 4.7 cm on day 7 (Figure 3a). Microscopically, the hyphae are hyalin, septate, and branched, forming intertwined mycelium tissues; Conidia are hyalin-shaped, cylindrical to oval, with rounded to slightly tapered ends and smooth walls (Figure 3b). Based on these macroscopic and microscopic characters, the isolate was morphologically identified up to the genus level as *Colletotrichum* sp.



Figure 3. Macroscopic (a) and microscopic (b) morphology of *Colletotrichum* sp. on PDA on day 7. Microscopic observations at 400× total magnification showed hyaline septate hyphae and cylindrical to oval conidia.

Conidia density of *Trichoderma* sp. Taratara isolate

The density of *Trichoderma* sp. conidia was calculated using a hemocytometer as many as three replicas with observations on five counting boxes (A–E) in two counting fields. In the first test, the average number of conidia was 116.60 conidia, the second test was 112.80 conidia, and the third test was 118.60 conidia. Based on the three replicas, an overall average of 116.00 conidia was obtained. Based on the average value of the number of conidia obtained, the conidia density calculation was carried out showing that the conidia density obtained was . This value showed that in every milliliter of suspension there were about 29 million conidia (Table 1). 2.9×10^7 konidia mL⁻¹

Table 1. Conidial density of *Trichoderma* sp. isolate Taratara

Replicate	Mean conidial count
1	116.60
2	112.80
3	118.60
Overall red	116.00
Conidial density	2.9×10^7 conidia mL ⁻¹

Viability Test of *Trichoderma* sp. Taratara Isolate Fungus

The viability of conidia on three consecutive repetitions (n = 3) was 97.92%; 92,31%; and 100.00%. The highest viability value was obtained in repeat III, while the lowest value was found in repeat II. The average viability of conidia obtained was $96.74 \pm 3.98\%$ (n = 3). Overall, a total of 103 conidia were observed in all three replicas, consisting of germinated and non-germinated conidia. The results showed that most conidia were able to germinate after 24 hours of incubation (Table 2).

Table 2. Conidial viability of *Trichoderma* sp. isolate Taratara (g = germinated conidia, u = non-germinated conidia, V = viability)

Replicate	g	u	Calculation	Viability (%)
I	47	1	$47/(47+1) \times 100$	97.92

II	24	2	$24/(24+2) \times 100$	92.31
III	29	0	$29/(29+0) \times 100$	100.00
Red $\pm$ SD				96.74 $\pm$ 3.98

Colony growth and antagonist activity

In one culture, *Trichoderma* sp. grew consistently faster than *Colletotrichum* sp. during the seven-day observation period. On the first day, the diameter of the colony of *Trichoderma* sp. had reached 2.1 cm compared to 0.9 cm for *Colletotrichum* sp.; on the 7th day, *Trichoderma* sp. reached 8.5 cm, nearly covering the PDA plate by 9 cm, while *Colletotrichum* sp. reached only 4.7 cm (Table 3).

Table 3. Colony diameter (cm) of *Trichoderma* sp. and *Colletotrichum* sp. in single culture over seven days of incubation

Fungus	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
<i>Trichoderma</i> sp.	2.1	5.2	6.1	7.6	8.1	8.4	8.5
<i>Colletotrichum</i> sp.	0.9	1.4	2.0	3.1	3.7	4.1	4.7

In the double culture test, the diameter of *Colletotrichum* sp. facing *Trichoderma* sp. (d2) was consistently smaller than the diameter of *Colletotrichum* sp. at control (d1) from the first day of observation. On the first day, d1 and d2 were 0.94 ± 0.09 cm and 0.84 ± 0.11 cm, respectively; on day 7, d1 increased to 4.66 ± 0.36 cm, while d2 reached only 1.82 ± 0.33 cm, after growth slowed since day 4 (Table 4). Growth inhibition of *Colletotrichum* sp. by *Trichoderma* sp. increased progressively during the incubation period, from $12.52 \pm 8.47\%$ on the first day to $60.43 \pm 9.64\%$ on the 7th day, with a slight decrease to $11.66 \pm 10.09\%$ on the second day before rising steadily thereafter (Table 4).

Table 4. Colony diameter of *Colletotrichum* sp. in the control (d1) and dual-culture treatment (d2), and the resulting growth inhibition (%), over seven days of incubation. Values are mean $\pm$ SD (n = 5).

São	D1 – Control (cm)	D2 – Dual Culture (cm)	Growth inhibition (%)
1	0.94 ± 0.09	0.84 ± 0.11	12.52 ± 8.47
2	1.38 ± 0.08	1.22 ± 0.16	11.66 ± 10.09
3	1.98 ± 0.15	1.60 ± 0.20	19.44 ± 10.60
4	3.06 ± 0.05	1.92 ± 0.28	37.20 ± 9.41
5	3.70 ± 0.14	1.90 ± 0.27	48.46 ± 8.73
6	4.14 ± 0.18	1.82 ± 0.33	55.77 ± 9.51
7	4.66 ± 0.36	1.82 ± 0.33	60.43 ± 9.64

Antagonist mechanisms

The competition mechanism is the earliest mechanism that occurs in *the dual culture method*. Based on the results of observations in this study, the mechanism that is directly observed is competition. It can be seen that *Trichoderma* sp. grows faster than *Colletotrichum* sp. so that it is able to dominate almost all media surfaces. This growth pattern shows that there is competition in the use of growing space, while the limitation of nutrient sources is a possibility that can support the inhibition of growth of *Colletotrichum* sp. (Figure 4).

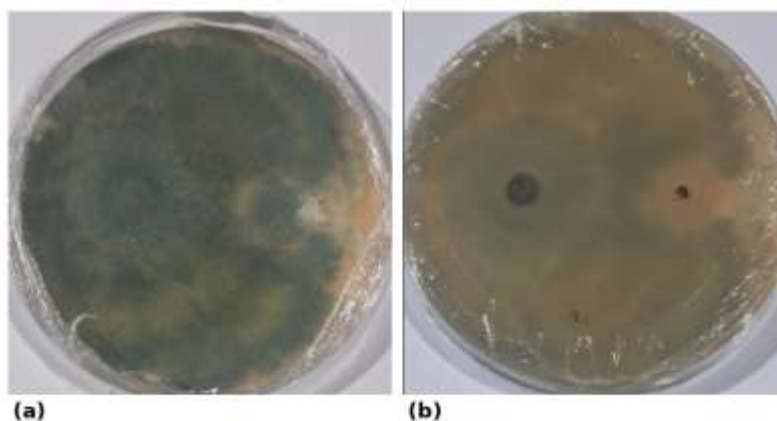


Figure 4. Dual-culture antagonistic assay between *Trichoderma* sp. isolate Taratara and *Colletotrichum* sp. on day 7: (a) front view; (b) back view, showing inoculation points 3 cm apart.

Microscopic observations showed that there was contact between the hyphae of *Trichoderma* sp. and *Colletotrichum* sp. in the interaction zone. Based on the observation results, there were hyphae structures that appeared to be intersecting and in some parts there were hyphae surrounding other hyphae. The identification of the two hyphae was based on the origin of the isolate used and the morphological character of each fungus that had been observed previously. The presence of contact and suspected hyphal coiling in the interaction zone can be an indication of the occurrence of an antagonistic mechanism in the form of mycoparasitism (Figure 5)



Figure 5. Microscopic observations of the interaction zone () showed hyphatic contact between *Trichoderma* sp. and *Colletotrichum* sp. on day 7 (magnification 400×).

Conclusion

Local isolates of *Trichoderma* sp. from Taratara Village, North Sulawesi, showed strong potential as a biological control agent against *Colletotrichum* sp., the causative agent of anthracnose chili. This isolate combines rapid vegetative growth, high conidium density (2.9×10^7 conidia mL⁻¹), and high conidium viability ($96.74 \pm 3.98\%$) with a progressive suppression of *Colletotrichum* sp. growth in multiple cultures, reaching resistance of $60.43 \pm 9.64\%$ on day 7. This antagonistic activity is mainly due to competition for planting space, as *Trichoderma* sp. consistently outperforms *Colletotrichum* sp. and eventually dominates the culture plate, along with direct contact of hyphae and possible windings in the interaction zone; antibiotics could not be confirmed, as no antifungal metabolite production tests were performed. Overall, these findings suggest that Taratara isolate is highly adapted to local conditions and is a promising and environmentally friendly candidate for developing site-specific biocontrol agents against chili anthracnose in North Sulawesi. Further research is needed to confirm its antibiotic-related mechanisms, evaluate its effectiveness under field conditions, and assess its compatibility with existing chili cultivation practices.

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